SciVerse ScienceDirect

Procedia Food Science 1 (2011) 1910 – 1917

Procedia
Food Science

11th International Congress on Engineering and Food (ICEF11)

Effect of pre-crystallization process and solid particle addition on cocoa butter crystallization and resulting microstructure in chocolate model systems

Lina Svanberg[a, b] *, Lilia Ahrné[a], Niklas Lorén[a], Erich Windhab[b]

[a] *SIK- the Swedish Institute for Food and Biotechnology, Box 5401, SE-402 29 Gothenburg, Sweden*
[b] *Swiss Federal Institute of Technology, Zurich, Institut für Lebensmittelwissenschaften, LFO E 12.1,ETH Zentrum, CH-8092 Zürich, Switzerland*

Abstract

The kinetics of cocoa butter crystallisation during solidification and resulting compactness of structure during storage for different chocolate model systems were investigated with respect to solid particle addition (sugar and cocoa particles) and pre-crystallization process (seeded/non-seeded). Confocal laser scanning microscopy (CLSM) was used to monitor microstructural evolution during solidification and image analysis were applied in order to quantify the kinetics. In order to quantify the compactness of structure during storage the migration rate of small-molecules was measured at different length scales. On the meso-scale, FRAP (Fluorescence Recovery After Photobleaching) was utilized to quantify local migration rate solely in the fat phase, whilst HPLC (High Performance Liquid Chromatography) measurements were performed to assess the global migration of same molecules on a macro scale. Both techniques were used in combination with microstructure characterization using CLSM and supported by differential scanning calorimeter melting curves for estimating cocoa butter polymorphism. During solidification, seeded samples tended to form multiple nucleation sites, inducing rapid growth of a crystal network. The non-seeded samples showed an altering structure, with some domains developing large spherical crystals while in other domains a more heterogeneous microstructure resulted. For the non-seeded samples, the impact of solid particles on the crystallization kinetics was also most pronounced. Both FRAP and HPLC analysis proved to generate relevant information of the effect of pre-crystallization and solid particles on compactness of structure during storage. FRAP-measurements gave detailed information of the hetero- or homogeneity in microstructure within the cocoa butter whilst the HPLC clearly showed the impact of solid particles. The combination of the two techniques revealed that a compact and homogeneous structure obtained through fast crystallization during solidification is required in order to retard global migration in confectionery systems.

Selection and/or peer-review under responsibility of 11th International Congress on Engineering and Food (ICEF 11) Executive Committee.

Keywords: Chocolate; Cocoa butter; Microstructure; Crystallisation; Processing

* Corresponding author. +46-10-516 66 00; fax: +46-31-83 37 82.
E-mail address: ls@sik.se

doi:10.1016/j.profoo.2011.09.281

1. Introduction

Fat bloom is a significant problem to the confectionary industry and is manifested as a greyish haze on the chocolate surface[1]. Filled confectionary products where the centre contains large amounts of highly mobile triacylglycerols (TAGs) are particularly sensitive to this phenomenon, as that filling fat migrates through the shell towards the surface where it can cause uncontrolled crystallisation and subsequently fat bloom. Additional quality defects associated with fat migration are shell softening, filling hardening, and sensory deterioration [2-4]. Chocolate has a complex microstructure in which sugar and cocoa particles are dispersed in a continuous phase of crystalline and liquid fat. Furthermore the crystalline cocoa butter can exist in six different polymorphic forms (often denoted by roman numbers I to VI and the Greek letters α, β and β´). To retard migration, it is crucial that the cocoa butter is in a stable crystal form (β_V) together with a low fraction of liquid TAGs[5]. During chocolate manufacturing, the most common procedure for obtaining a stable β_V form involves subjecting the chocolate to a well-defined temperature programme under the action of shear. However, in 2000, Zeng presented a novel pre-crystallisation technique, "seeding technique", for producing well-tempered chocolate by homogeneously mixing 0.2-2% (w/w) of cocoa butter crystals in the most stable form β_{VI} with pre-cooled chocolate[6].

Confocal laser scanning microscopy (CLSM) offers the possibility to monitor microstructural development at different depths and length scales in the bulk under dynamic conditions which enables the survey of cocoa butter crystallisation. The technique can also be combined with fluorescence recovery after photobleaching (FRAP). Using the FRAP technique in combination with CLSM, it is possible to measure the mobility of fluorescent molecules directly in the microscope [7] and it has recently been used to correlate small-molecular diffusion through a cocoa butter matrix with the structural characteristics of the matrix [8]. In FRAP, first part of the fluorescent molecules within a well-defined area of the sample is deactivated using a high intensity laser beam that results in less intensity in the bleached area. Second, the fluorescent recovery, i.e., diffusion of unbleached molecules into the bleached area and simultaneous diffusion of bleached molecules into the surroundings, is detected as an increase in intensity in the bleached area [9, 10]. Parameters frequently derived from FRAP measurements are the mobile fraction and rate of mobility of the fluorescent molecules [11]. Consequently, FRAP offers the possibility to measure local diffusion of small florescence molecules within a structure. On a global scale, fat migration has previously been measured with high-performance liquid chromatography (HPLC) [12], flat bed scanner [13] or magnetic resonance imaging [14].

The objective of this work was to investigate the effect of improved/sub-optimal pre-crystallisation as well as impact of solid chocolate ingredients (sugar and cocoa particles) on the microstructure during cooling and storage in chocolate model systems. The CLSM technique was applied to follow fat crystallisation during cooling and the resulting microstructure was quantified using FRAP and HPLC during storage by measuring local and global diffusion properties of small molecules.

2. Materials and Methods

Chocolate model systems were created by adding sugar or cocoa particles to cocoa butter according to the method described by [15]. To avoid particle density effects (as sugar is present in larger quantities than cocoa particles in commercial chocolate), the mass ratio between cocoa butter and solid particles was set to 2:1 with a total sample weight of 0.54 g. To visualise the crystallisation process with CLSM and enabling FRAP measurements the fluorescent labelled non-polar fatty acid analogue BODIPY FL C_{16} (Invitrogen Ltd., Paisley, UK) was mixed with the chocolate model systems while melted at a final concentration of 37 ppm. Subsequently, the fluorescent signal from BODIPY could be deactivated by a high intensity laser beam, thereby also enabling FRAP measurements. All model systems were subjected to one of two pre-crystallisation procedures: seeded or non-seeded, corresponding to improved and sub-optimal processing followed by 60 minutes of cooling at 14°C according to the method described by [15].

The crystal growth in chocolate model systems during cooling at 14˚C was followed using a Leica TCP SP2 confocal laser scanning microscope, (Mannheim, Germany). Images at a central point of each sample were taken with 1-2 minute interval using an HCX PL AP0 oil objective with 63 times magnification and a numerical aperature of 1.4. All images were recorded at a resolution of 1024×1024 pixels.

After 60 minutes of cooling at 14˚C, FRAP measurements were made using a Leica TCP SP2 confocal laser scanning microscope (Mannheim, Germany) together with the rFRAP application available on the Leica software according to the procedure described by [16]. The size of the ROI was set to a 10 × 10 μm square, according to the rectangular FRAP (rFRAP) model developed by [17]. This relatively small ROI allowed measurement of the local diffusion rate of small molecules, i.e. BODIPY, solely in the fat crystal structure, so the ROI was always placed on the fat crystal networks visible in the frames. For each replicate in the model system, five rFRAP measurements were made immediately after 60 min of cooling and after one week of storage at 15°C. Only the rFRAP measurements where the experimental and model data coincided were included in the results. Calculations of the local diffusion rate of BODIPY, expressed in $\mu m^2/s$, in the cocoa butter matrix were then made according to the methods of rectangular FRAP [17].

Global diffusion properties of the small molecule (BODIPY) were assessed with HPLC. The chocolate model systems used for HPLC analysis were either pure cocoa butter or cocoa butter mixed with both sugar and cocoa particles. Samples were also produced in which un-tempered cocoa butter (melted at 49°C for 10 min then cooled to 14°C for 60 min) and mixed with BODIPY to a final concentration of 100 ppm. Immediately after cooling, three samples representing 1) seeded/non-seeded cocoa butter, 2) un-tempered cocoa butter mixed with BODIPY, and 3) seeded/non-seeded cocoa butter, sugar, and cocoa particles, were placed into contact and held together by plastic foil. The samples were stored at 15°C for 5 d to allow BODIPY from the central layer to migrate into the adjacent model systems. After 5 d of storage, the three-layer systems were split to their original layers using a razorblade. Each separated model system was thereafter sliced into 8 thin layers (160 μm thick) using a Leica CM1900 cryostat (Mannheim, Germany). Every second slice was collected for HPLC analysis corresponding to original distances of 320, 640, 960, and 1280 μm from the cocoa butter/BODIPY layer. The amount of BODIPY in each slice was determined with HPLC according to the method described by [16]. All three-layer model systems were produced in duplicates.

3. Results and Discussion

3.1 Microstructure during cooling and storage

CLSM micrographs illustrating the time-dependent structure evolution of seeded and non-seeded pure cocoa butter, cocoa butter and sugar and cocoa butter and cocoa particles are presented in Figure 1 and are adaptations of what has previously been published in [15, 16]. The staining dye (BODIPY) exhibited significantly higher affinity to the liquid cocoa butter compared to the crystals, thereby enabling the two phases to be distinguished by negative contrast. Both the sugar and cocoa particles appeared as black areas in the micrographs.

The structure formed during 60 minutes of cooling differed substantially between the two pre-crystallisation techniques. All seeded samples in Figure 1 formed multiple nucleation sites, which induced rapid growth of crystals within the first 15 minutes of cooling. The seeds were easily mixed in the samples, which resulted in a homogenous microstructure, with small proportions of liquid fat trapped within the fat crystal network. A more heterogeneous microstructure was observed in the non-seeded samples which coincide with previous reports, where the microstructure of poorly tempered chocolate has been investigated with various microscopy techniques [18-20]. The non-seeded samples containing cocoa

particles formed small rod-shaped crystals (Figure 1(c)) which differed significantly from the homogenous microstructure formed in corresponding seeded samples where no such crystal morphology was detected. Further results can be observed in Svanberg et al., 2010 [15].

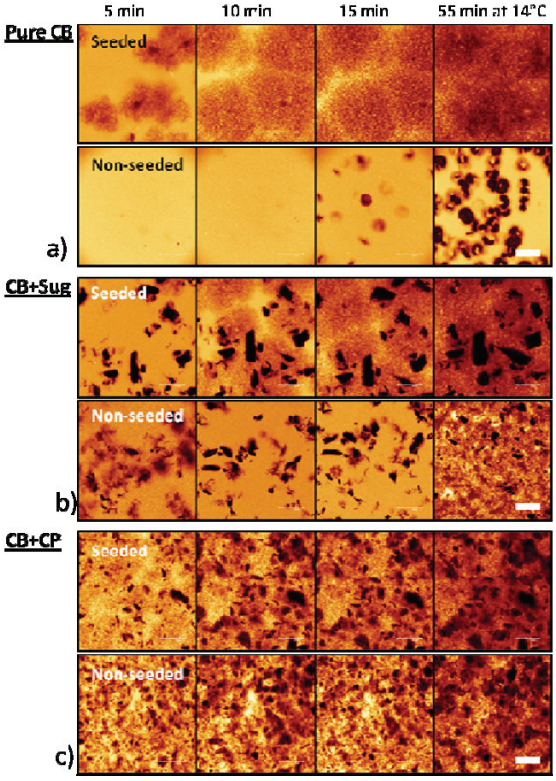

Fig. 1. CLSM micrographs showing the time-dependant crystal growth in seeded and non-seeded (a) pure cococa butter (CB), (b) cocoa butter and sugar (CB+Sug) and (c) cocoa butter and cocoa particles (CB+CP), during cooling at 14˚C for 55 minutes. Scale bars represent 25 μm

During storage, extensive post-crystallisation occurred in the non-seeded samples and after one week the microstructure largely resembled the seeded samples. This is illustrated in Figure 2 where CLSM micrographs of the microstructure in seeded and non-seeded model systems after one week of storage are displayed.

3.2 Structure density during storage

Immediately after 60 min of cooling, where significant differences in microstructure were observed between the seeded and non-seeded samples, diffusion measurements with FRAP and HPLC were initiated to correlate the quantification of structure density with the results of the microstructure characterization. The results presented here are adaptations of what has been published in [16].

The calculated local diffusion rates of BODIPY, obtained from FRAP measurements, for all chocolate model systems after 60 min of cooling and after one week of storage are presented in Figure 3. Directly after cooling, the non-seeded samples had significantly higher local diffusion rates of BODIPY compared to the seeded samples. However, for the non-seeded sample with CB+sug the standard deviation was too

large for any statistical conclusions. Initially, the substantial error bars in the non-seeded samples indicate larger variations in microstructure in the fat phase. This also agrees with previous findings regarding the compactness of structure density found in properly/improperly traditionally pre-crystallized chocolate[1, 2]. The seeded samples had markedly low local diffusion rates of BODIPY; however, it is important to note that all these samples are close to the detection limit for the applied FRAP method, which is 0.1μm^2/s [17]. After one week of storage, the differences between the seeded and non-seeded samples were less pronounced, as the local diffusion rate of BODIPY in the non-seeded samples had decreased significantly (Fig. 2(b)). The seeded samples displayed no such alteration during storage and the local diffusion rate remained constantly low. Furthermore, no significant differences in local diffusion rate were observed between chocolate model systems subjected to the same pre-crystallisation process i.e. possible effects of solid particles on structure density in the fat phase could not be significantly distinguished using the FRAP technique.

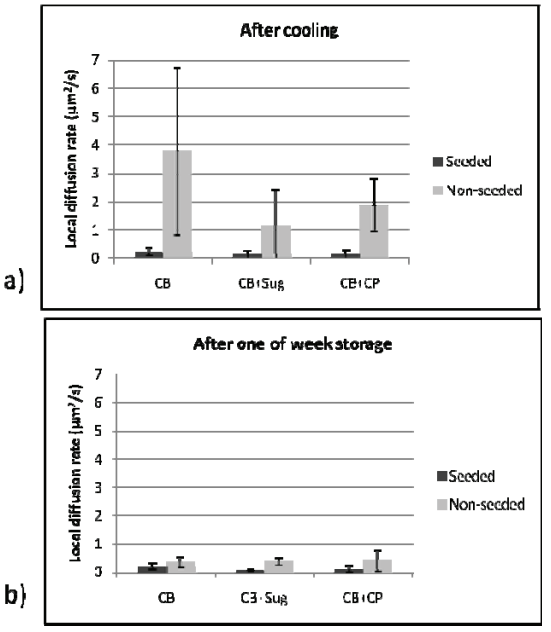

Fig. 2. Calculated local diffusion rate of BODIPY (μm^2/s) in seeded versus non-seeded chocolate model systems immediately after cooling (a) and after one week of storage at 15°C (b). The error bars represent the standard deviation of fifteen FRAP measurements divided on three sample measurements

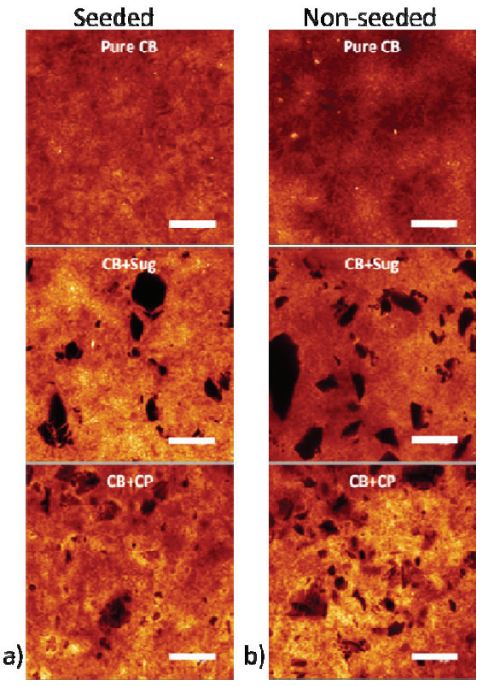

Fig. 3. Representative CLSM micrographs of the microstructure in seeded (a) and non-seeded (b) chocolate model systems after one week of storage at 15°C. Scale bars represent 25 μm

To further quantify the structure density in the various microstructures created by pre-crystallisation and/or solid particle addition, the global diffusion properties of BODIPY were determined using HPLC. A high capacity to retard diffusion was treated as equivalent to a dense structure. The amount of BODIPY, expressed in μg of BODIPY/mg sample, as a function of distance from the cocoa butter/BODIPY slice is presented in Figure 4. A more detailed description of the structure density results can also be found in [16].

In the area nearest the cocoa butter/BODIPY slice, the amount of BODIPY was higher in the non-seeded samples for both pure cocoa butter and samples with solid particles (Figure 4(a)). This indicates

that the microstructure in these samples had less ability to withstand the diffusion of BODIPY. Furthermore, when comparing samples subjected to same pre-crystallisation, the addition of solid particles tended to retard the global diffusion. This could be due to an increase in tortuosity, i.e., deviation from the linear diffusion path as the solid particles forced the BODIPY to diffuse round them. This also agrees with previous reports examining the diffusion properties of various filling fats through a chocolate shell in the presence and absence of solid particles [2, 21, 22].

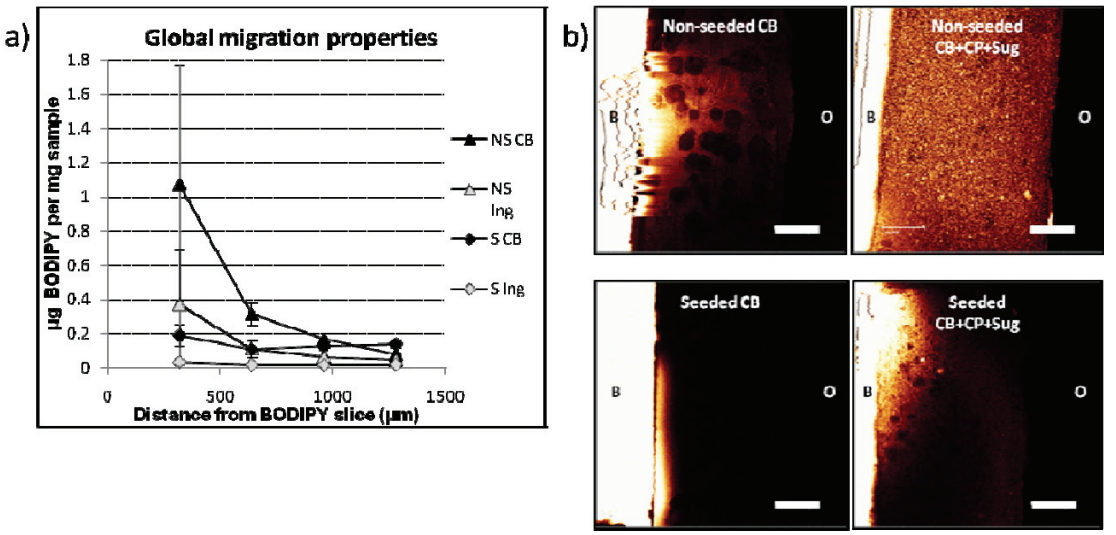

Fig. 4. (a) Amount of BODIPY (µg of BODIPY/mg sample), measured with HPLC, as a function of distance from the cocoa butter/BODIPY slice. Error bars represent the standard deviation of two sample replicates. (b) Low-magnification CLSM micrographs of the global diffusion of BODIPY from the cocoa butter/BODIPY slice [B] into the model systems. The outer surface is indicated by [O] and scale bars represent 500 µm

Low-magnification CLSM micrographs of the three-layer systems after 5 d of storage are presented in Figure 4(b). The bright area to the left in each micrograph represents to the cocoa butter/BODIPY slice, indicated by [B]. The investigated chocolate model systems correspond to the adjacent dark or orange area to the right, which is visible only due to diffusion of BODIPY. The outer surface, [O], was completely black in all the micrographs, and the solid particles appeared as dark spots in the CB+CP+Sug layer (Figure 3(b)). Since all the micrographs presented were taken using the same laser intensity, the significantly darker micrographs of the seeded samples correspond to less diffusion of BODIPY. Larger amounts of BODIPY had migrated through the non-seeded samples, which were manifested as bright/white areas in the micrographs.

From the microstructure characterization with CLSM it was found that post-crystallisation in the cocoa butter compensated for the initial heterogeneous microstructure found in the non-seeded samples. However, the first days with a less dense structure, when crystallisation was not completely developed, were enough to allow significantly higher levels of diffusion to occur. It can be concluded that a dense structure is required immediately after cooling to retard diffusion and this is special relevant in filled chocolate products.

4. Conclusions

The CLSM was found to be a good technique for monitor the cocoa butter crystallisation in chocolate model systems during cooling. Seeded samples formed multiple nucleation sites, which induced a rapid growth of crystals and resulted in a more homogenous microstructure. The non-seeded samples showed a more random structure, with some areas developing large spherical crystals while other parts gained a more heterogeneous microstructure with large inclusions of liquid fat and small compact crystals.

The structure density in the various model systems during storage could be quantified by small-molecule diffusion at different length scales using FRAP and HPLC. FRAP measurements gave detailed information on the heterogeneity or homogeneity of microstructure in the cocoa butter, whereas the HPLC indicated the impact of solid particles. Both techniques showed significant differences in terms of structure density between the pre-crystallisation processes, seeded and non-seeded. During storage, the non-seeded samples experienced extensive post-crystallisation and evolved towards the dense structure found in the seeded samples. However, the initial less compact structure in the non-seeded samples created a more favourable environment in which the global diffusion could occur.

References

[1] R.W. Hartel, 1999, Chocolate: Fat Bloom During Storage, The Manufacturing Confectioner. **79**(5): p. 89-99.

[2] V. Ghosh, G.R. Ziegler and R.C. Anantheswaran, 2002, Fat, Moisture and Ethanol Migration through Chocolates and Confectionary Coatings, Critical Reviews in Food Science and Nutrition. **42**(6): p. 583-626.

[3] G. Talbot, 1989, Fat migration in confectionery products, Confectionery Production. **55**: p. 655-656.

[4] G. Ziegleder, 1997, Fat migration and bloom, The Manufacturing Confectioner. **77**(2): p. 43-44.

[5] G.R. Ziegler, A. Shetty and R.C. Anantheswaran, 2004, Nut Oil Migration Through Chocolate, The Manufacturing Confectioner. **84**(9): p. 118-126.

[6] Y. Zeng, *Impf- und Scherkristallisation von Schokoladen. PhD Thesis*, in *Laboratorium fur Lebensmittelverfahrenstechnik*. 2000, ETH-Eidgenössische Technische Hochschule Zürich.

[7] N. Lorén, M. Nydén and A.-M. Hermansson, 2009, Determination of local diffusion properties in heterogeneous biomaterials, Advances in Colliod and Interface Science. **150**: p. 5-15.

[8] S. Marty, M. Schroeder, K.W. Baker, G. Mazzanti and A.G. Marangoni, 2009, Small-Molecule Diffusion through Polycrystalline Triglyceride Networks Quantified Using Fluorescent Recovery after Photobleaching, Langmuir. **25**(15): p. 8780-8785.

[9] D. Axelrod, D.E. Koppel, J. Schlessinger and E. Elson, 1976, Mobility measurement by analysis of fluorescence photobleaching recovery kinetics, Biophysical Journal. **16**: p. 1055-1069.

[10] R. Peters, J. Peters, K.H. Tews and W. Bähr, 1974, A microfluorimetric study of translational diffusion in erythrocyte membranes, Biochimica et Biophysica Acta. Biomembranes. **367**: p. 282-294.

[11] E.A.J. Reits and J. Neefjes, 2001, From fixed to FRAP: measuring protein mobility and activity in living cells, Nature Cell Biology. **3**: p. E145-E147.

[12] R.S. Khan and D. Rousseau, 2006, Hazelnut oil migration in dark chocolate- kinetic, thermodymnamic and structural considerations, European Journal of Lipid Science and Technology. **108**: p. 434-443.

[13] S. Marty, K. Baker and A.G. Marangoni, 2009, Optimization of a scanner imaging technique to accurately study oil migration kinetics, Food Research International. **42**(3): p. 368-373.

[14] M.E. Miquel, S. Carli, P.J. Couzens, H.J. Wille and L.D. Hall, 2001, Kinetics of the migration of lipids in composite chocolate measured by magnetic resonance imaging, Food Research International. **34**: p. 773-781.

[15] L. Svanberg, L. Ahrné, N. Lorén and E.J. Windhab, 2010, Effect of Sugar, Cocoa Particles and Lecithin on Cocoa Butter Crystallisation in Seeded and Non-Seeded Chocolate Model Systems, Accepted for publication in Journal of Food Engineering.

[16] L. Svanberg, L. Ahrné, N. Lorén and E.J. Windhab, 2011, Effect of pre-crystallization process and solid particle addition on microstructure in chocolate model systems, Accepted for publication in Food Research International.

[17] H. Deschout, J. Hagman, S. Fransson, J. Jonasson, M. Rudemo, N. Lorén and K. Braeckmans, 2010, A Versatile Rectangle FRAP Method for Diffusion Measurements with a Laser Scanning Microscope, Biomacromolecules.

[18] E.O. Afoakwa, A. Paterson, M. Fowler and J. Vieira, 2008, Effects of tempering and fat crystallization behaviour on microstructure, mechanical properties and appearance in dark chocolate systems., Journal of Food Engineering. **89**: p. 128-136.

[19] A. Bowser, 2006, Crystallization of cocoa butter, The Manufacturing Confectioner. **86**(9): p. 115-118.

[20] Y. Kinta and R.W. Hartel, 2010, Bloom Formation on Poorly-Tempered Chocolate and Effects of Seed Addition., Journal of the American Oil Chemists' Society. **87**(1): p. 19-27.

[21] W.L. Lee, M.J. McCarthy and K.L. McCarthy, 2010, Oil Migration in 2-Component Confectionery Systems, Journal of Food Science. **75**(1): p. E83-E89.

[22] D. Rousseau, The microstructure of chocolate, in Understanding and controlling the microstructure of complex foods, D.J. McClements, Editor. 2007, Woodhead Publishing Limited: Cambridge. p. 648-687.

Presented at ICEF11 (May 22-26, 2011 – Athens, Greece) as paper FMS521.